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14. ABSTRACT

This report provides an update on the study of the role of polyamine oxidase(PAOh1/SMO, spermine oxidase) in drug response of human breast cancer. Thecytotoxic effects of two novel polyamine analogue compounds, CGC-11144 and CGC-11047 were evaluated in the human breast cancer cell lines MDA-MB-231, Hs578t,MCF-7, 468, and T47D. Additionally, the hypothesis that induction of spermineoxidase by pro-inflammatory agents plays a role in carcinogenesis was evaluatedusing a model system of exposure of MCF-10a human breast epithelial cells tocigarette smoke extract (CSE).

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INTRODUCTION

Polyamines are ubiquitously present in cells and are absolutely required for cell growth and differentiation (Figure 1a). Intracellular concentrations of these alkyl amines are tightly controlled; the disregulation of polyamine metabolism in cancer cells has long been recognized as a potential target for chemotherapeutic agents (Wang 2005). In particular, abherrant regulation of polyamine metabolism has been observed in breast cancer tissues, resulting in increased levels of intracellular polyamines and reexpression of spermine oxidase in response to specific polyamine analogues is a major determinant of drug response (Pledgie 2005). Spermine oxidase (PAOh1/SMO) is a newly-characterized member of the mammalian polyamine catabolic pathway. The products of its activity are the polyamine spermidine, 3-aminopropanal, and the reactive oxygen species, H_2O_2.

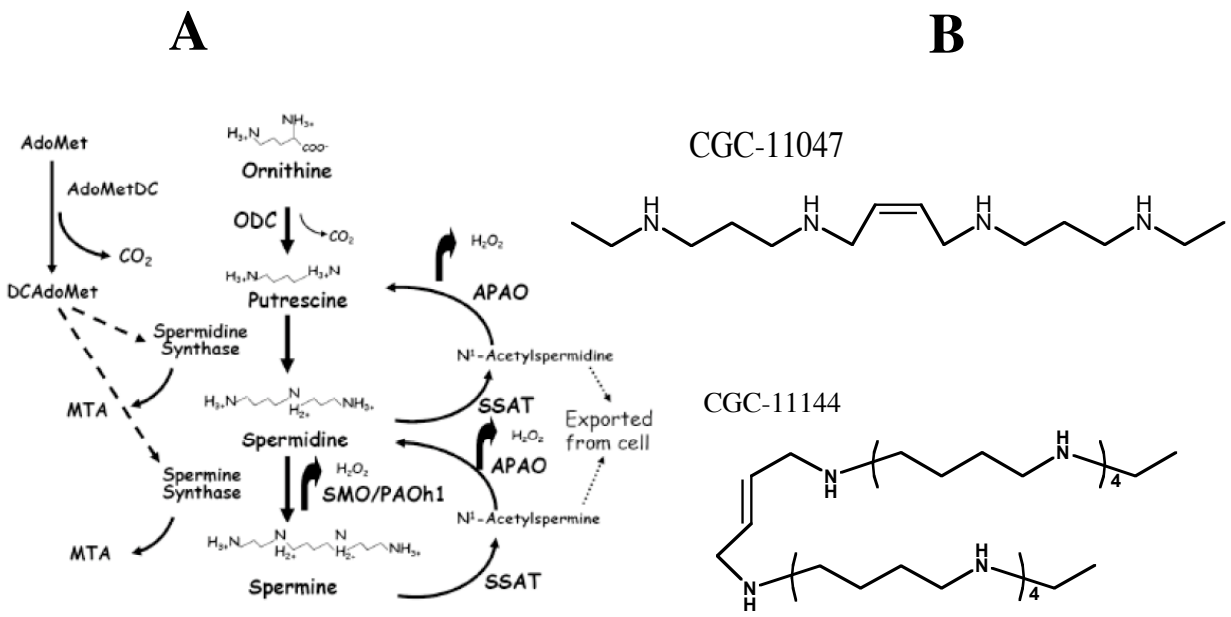

Figure 1. The polyamine metabolic pathway. Abbreviations: AdoMet, S-adenosylmethionine; DCAdoMet, decarboxylated S-adenosylmethionine; MTA, methyl-thioadenosine; ODC, ornithine decarboxylase; PAO, N^1-acetylpolyamine oxidase; SMO/PAOh1, spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase.

Previous work by our research group has shown that SMO can be induced by polyamine analogue treatment in a number of lung cancer cell lines, resulting in polyamine depletion, H_2O_2 production, growth inhibition, and promotion of apoptosis (Wang 2001, Devereux 2003, Huang 2005). Previous and ongoing work supported by this grant are focused on expanding the study to the effects of polyamine analogues on a number of breast cancer cell lines.

Additionally, recent studies strongly suggest that SMO plays a role in the process of inflammation-associated carcinogenesis (estimated at 20-30% of all epithelial cancers). Recently, we have made several key findings showing that exposure to inflammatory stimuli such as bacterial infection or pro-inflammatory

cytokines leads to the induction of SMO in stomach, lung, and colon cell lines (Babbar 2006a, Xu 2004, and unpublished observations). We have undertaken a collaborative pilot study to investigate whether the inflammatory microenvironment caused by exposure to breast cancer cells to cigarette smoke extract (CSE) results in increased SMO expression.

Improved understanding of the role of SMO in both inflammation-associated carcinogenesis and as a target for chemotherapy will contribute to our understanding of human breast cancer and aid in the development of potential novel therapies.

BODY

Previously, the effects of the polyamine analogue bis(ethyl)norspermine on the growth of the human breast cancer cell lines MDA-MB-231, Hs578t, MCF-7, and T47D was investigated. We have expanded our analysis of the above cell lines (as well as the 468 mammary carcinoma cell line) to include two second-generation polyamine analogue compounds, CGC-11144 and CGC-11047 (Figure 1b). Cells were seeded at 5000 per well in 96 well plates in quadruplicate and treated with either 11144 (for 96 hours) or 11047 (for 120 hours) in increasing doses ranging from 0.1-50 uM (11047) or 0.1-10 uM (11144). After 96 or 120 hours, MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide) assays were performed, absorbance at 540 nm recorded, and cell viability calculated.

Relative viability versus mock-treated cells was calculated for each cell line and dose and is shown in Figure 2. 11047 displayed moderate cellular toxicity in all five breast cancer cell line studied, while 11144 displayed greater than 90% cytotoxicity in 4/5 cell lines at 96 hr. Further experiments will evaluate the role of spermine oxidase induction in determining the susceptibility of the various cell lines to analogue-induced cytotoxicity. This will be carried out using both pharmacological inhibition of SMO with the specific polyamine oxidase inhibitor MDL 72,527 and RNAi-mediated knockdown of SMO expression. A tetracycline-inducible SMO overexpressing human breast cancer cell line will also be utilized to directly study the effects of increased SMO activity in the context of treatment with polyamine analogue compounds.

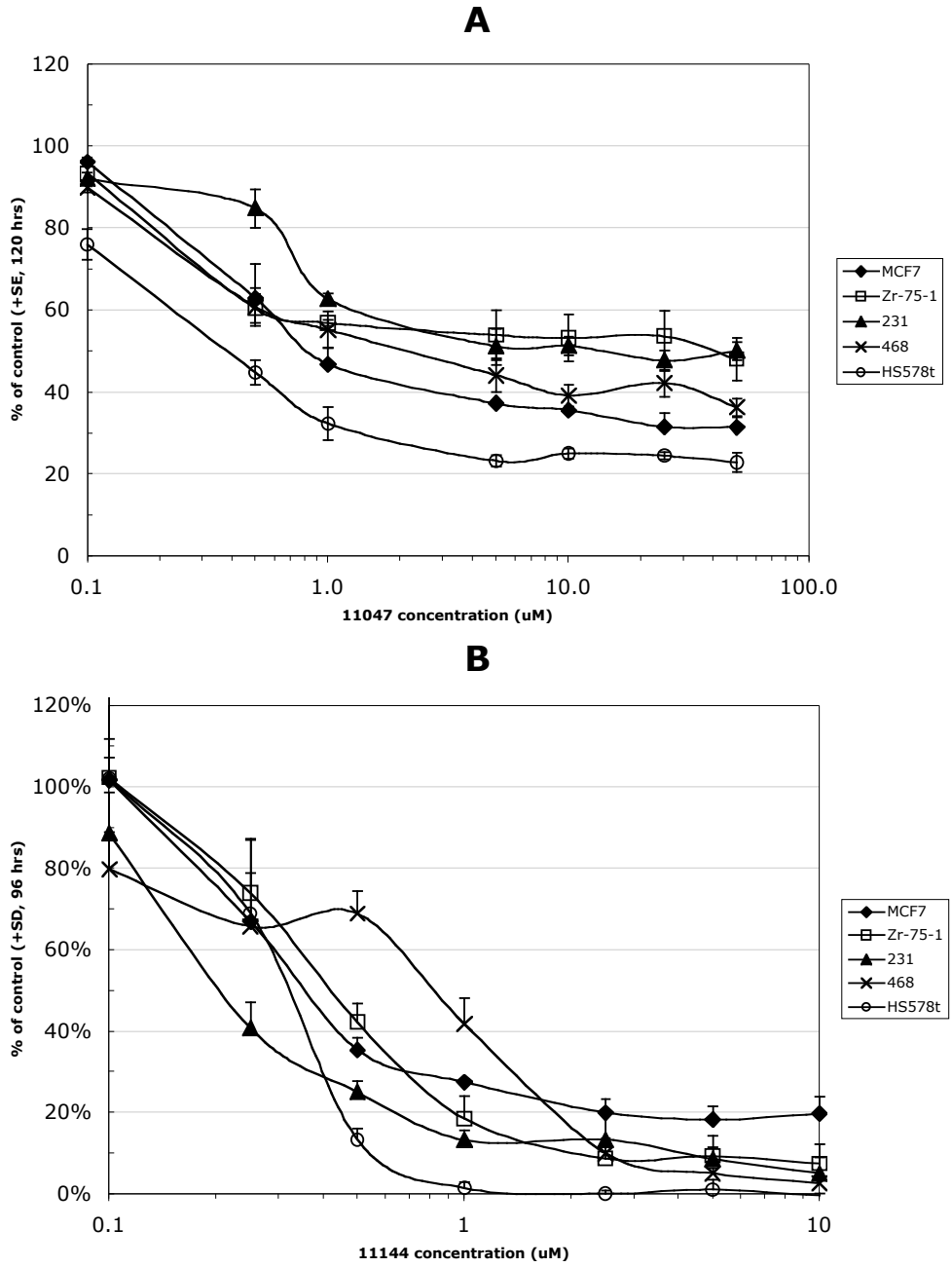

Figure 2. Inhibition of growth of human breast cancer cell lines by the polyamine analogue compounds **(A)** 11047 and **(B)** 11144 as measured by MTT assay.

To investigate the potential role of spermine oxidase (SMO) in inflammation-associated mammary carcinogenesis, the effect of cigarette smoke extract (CSE) exposure on non-tumorigenic MCF-10a human breast epithelial cell line was determined. MCF-10a cells were seeded, grown to 90% confluency, and then treated with culture media containing varying concentrations of CSE (mock, 0.01%, 0.1%, 0.5%, 1.0%) for 1,3, or 24 hours. Cell lysates from each time point were harvested and RNA was purified, Dnase-treated, reverse transcribed with murine leukemia virus reverse transcriptase and oligo-dT primers, and analyzed by quantitative real-time PCR. Additionally, cell lysates exposed to CSE for 1 hour were harvested in 0.083M glycine, pH 8.0 and spermine oxidase enzyme activity was measured via a chemiluminescence assay.

Quantitative real-time PCR results indicate moderate induction of spermine oxidase at all time points (1, 3, 24 hours), with maximum induction of SMO mRNA after 3 hr CSE exposure (Figure 3). Based on our previously published studies of lung carcinogenesis (Babbar 2006b) and preliminary data in prostate, colorectal and gastric carcinogenesis models, we also evaluated the effects of CSE on another key polyamine catabolic enzyme, spermine/spermidine N^1-acetyltransferase (SSAT). Treatment of MCF-10a cells with CSE resulted in a rapid 3-fold induction of SSAT mRNA at the one hour time point, with only moderate induction seen at 3 and 24 hour time points. Moderate induction of spermine oxidase enzyme activity was observed after 1 hour treatment of MCF-10a cells with CSE (Figure 4).

Continuing experiments will further expand our understanding of mechanism and significance of the induction of polyamine catabolism by CSE or other pro-inflammatory mediators in human breast cancer cell lines. We will further determine the extent of SMO induction at the levels of mRNA, protein, and enzyme activity at an expanded set of exposure lengths. The ability of CSE to induce downstream events, such as reactive oxygen species production and DNA damage, which may lead to carcinogenic mutations will also be studied.

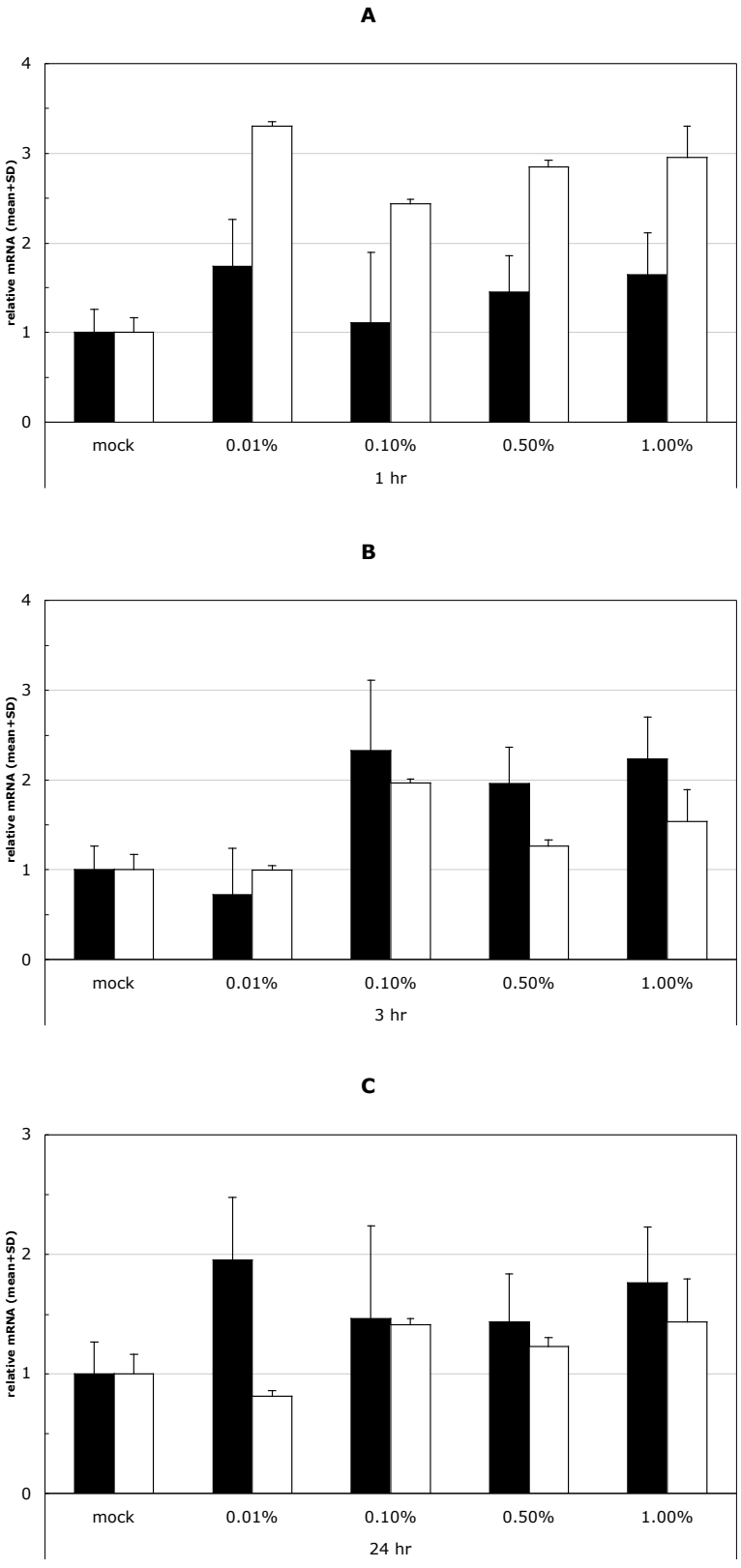

Figure 3. Induction of spermine oxidase (SMO, black bars) and spermine/ spermidine N1 acetyl-transferase (SSAT, open bars) in MCF-10a human breast cancer cells by cigarette smoke extract (CSE) at the indicated concentrations and lengths of exposure. Data from quantitative real-time PCR experiments performed in triplicate are normalized to GapDH expression and expressed as mean fold induction plus standard deviation.

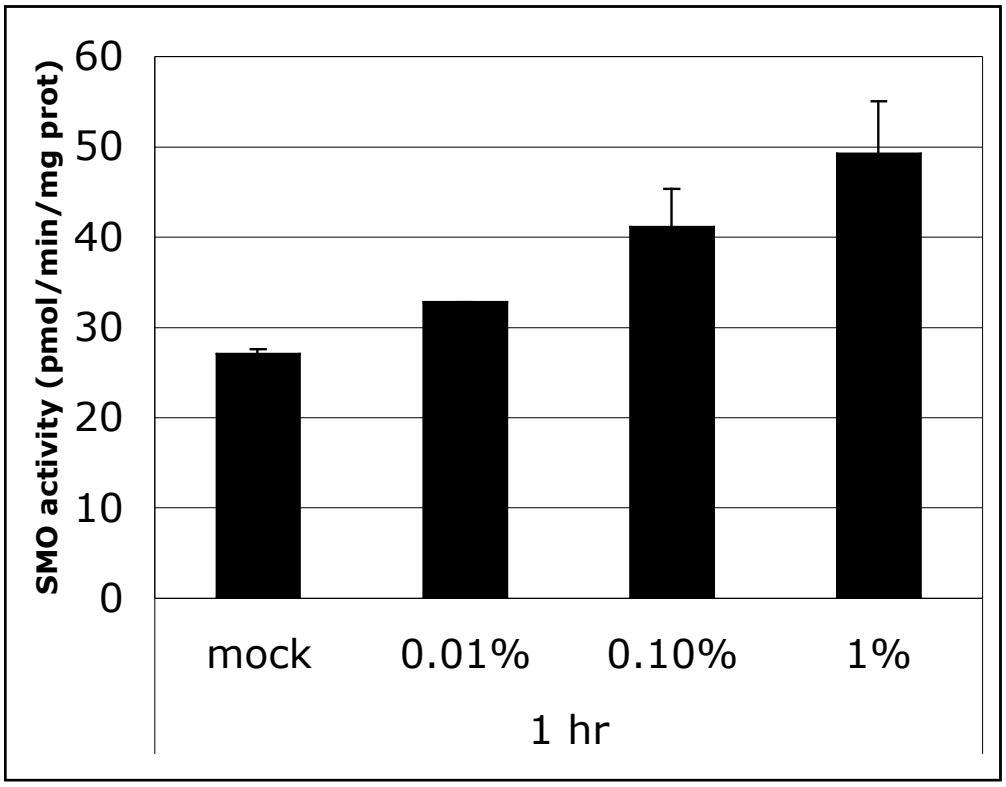

Figure 4. Induction of spermine oxidase enzyme activity by cigarette smoke extract (CSE). MCF-10a human breast epithelial cells were seeded, treated with the indicated concentrations of CSE, lysed at -80ºC in 0.083 M glycine, pH 8.0, and spermine oxidase enzyme activity was determined by a chemiluminescence assay. Enzyme activity (mean pmol/min/mg protein + SD) is presented for experiment performed in triplicate.

KEY RESEARCH ACCOMPLISHMENTS

- Two additional polyamine analogues, 11144 and 11047, were evaluated and found to be effective agents for the inhibition of growth of a library of human breast cancer cell lines.
- Exposure of the MCF-10a human breast epithelial cell line to cigarette smoke extract results in the rapid induction of the polyamine catabolic enzymes spermine oxidase and spermine/spermidine N1-acetyltranferase.

REPORTABLE OUTCOMES

- Talmesha Richards, the previous PI of this award applied for and was awarded a Department of Defense Predoctoral Traineeship Award to continue to pursue further breast cancer research with the assistance of preliminary data and experience gained from this award.
- Andrew Goodwin, the most recent PI of this award, has utilized the experience gained from this award to formulate a research proposal related to colorectal carcinogenesis and has applied for a Graduate Student Award from the American Gastroenterology Association.
- Talmesha Richards and Andrew Goodwin have both applied knowledge and experience gained from this award to apply for and be accepted to attend and present their research at the 2007 Gordon-Kenan Graduate Research Seminar on Polyamines and the 2007 Gordon Research Conference on Polyamines in Waterville Valley, NH.

CONCLUSIONS

Work in our research group, including that supported by this grant, has shown that the polyamine catabolic enzyme spermine oxidase (SMO) is of tremendous interest and importance in the field of cancer research. Our published and preliminary data show that SMO-mediated production of hydrogen peroxide is a key factor in the cytotoxic effects of anti-tumor polyamine analogues. Further, SMO appears to play a role in inflammation-associated epithelial carcinogenesis via production of reactive oxygen species and DNA damage.

REFERENCES

Babbar, N. and Casero, R. Jr. 2006. *Cancer Research*. 66: 11125-11130.

Babbar, N., Hacker, A., Huang, Y., and Casero, R. Jr. 2006. *Journal of Biological Chemistry*. 281: 24182-24192.

Devereux W, Wang Y, Stewart TM, Hacker A, Smith R, Frydman B, Valasinas AL,

Reddy VK, Marton LJ, Ward TD, Woster PM, Casero RA. Jr. 2003. *Cancer Chemotherapy and Pharmacology*. 52: 383-390.

Huang, Y., Pledgie, A., Casero, R. Jr, and Davidson, N. 2005. *Anti-Cancer Drugs*. 16: 229-241.

Pledgie, A., Huang, Y., Hacker, A., Zhang, Z., Woster, P., Davidson, N., Casero, R. Jr. 2005. *Journal of Biological Chemistry*. 280 (48): 39843-39851.

Wang, Y. and Casero, R. 2005. *Journal of Biochemistry*. 139: 17-25.

Wang, Y., Devereux, W., Woster, P., Stewart, T., Hacker, A., Casero, R. Jr. 2001. *Cancer Research*. 61: 5370-5373.

Xu, H., Chaturvedi, R., Cheng, Y., Bussiere, F., Asim, M., Yao, M., Potosky, D., Metzler, S., Rhee, J., Kim, S., Moss, S., Hacker, A., Wang, Y., Casero, R. Jr., Wilson, K. 2004. *Cancer Research*. 64: 8521-8525.